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Tubakia koreana sp. nov. causing *Quercus* leaf blight

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Abstract — A new species, *Tubakia koreana* (*Diaporthales*, *Ascomycota*), isolated in Korea from blight symptoms on *Quercus* leaves, is described and illustrated. The species is morphologically distinct from other *Tubakia* species and causes a progressive necrotic leaf blight that differs from leafspot symptoms caused by other species. Phylogenetic analysis of ITS sequences supports a monophyletic *T. koreana* clade independent of other *Tubakia* species.

Key words — leaf death, *Quercus acutissima, Quercus mongolica*, sawtooth oak

Introduction

Oaks (*Quercus* spp.), which are widely distributed in the northern hemisphere, are the most common tree species in Korea, occurring in ~27% of the mountainous area. Four species of *Tubakia* have been reported in Korea: *T. japonica* (Sacc.) B. Sutton (on *Castanea crenata*, *Quercus acutissima*, and *Q.* × *alienoserratoides*), *T. rubra* (T. Yokoy. & Tubaki) B. Sutton (on *Q. serrata*), and an undescribed *Tubakia* sp. (on *Q. rubra*; Cho & al. 2004); and *Tubakia seoraksanensis* H.Y. Yun (on *Q. mongolica*; Yun & Rossman 2011). In search of more *Tubakia* species, we surveyed various regions in Korea; in the mountain localities of several South Korean provinces leaf blight symptoms were observed from September 2009 through November 2014 on four species: *Q. acutissima*,

Q. × *alienoserratoides, Q. mongolica*, and *Q. serrata*. There had been no previous report of severe foliar blight symptoms of oaks in Korea. The symptoms were similar to those of bur oak blight in U.S.A., caused by *T. iowensis* T.C. Harr. & McNew (Harrington & al. 2012). In this study, the causal agent of the Korean oak leaf blight was examined to determine its morphological and molecular genetic characteristics. It was compared with other *Tubakia* species for identification of the fungus, which is described below as *T. koreana*.

Materials & methods

For fungal isolation, conidiomata on leaves of *Quercus* spp. with blight symptoms were detached from the adaxial leaf areas along the necrotic mid- and lateral veins and cultured on malt extract agar (MEA, 1.5% malt extract, and 2% agar) at 25°C in the dark. Fungal hyphae growing out of the conidiomata were harvested and sub-cultured on fresh MEA at 25°C for 14 days, at which time mycological characteristics were examined. For detailed morphological study, microscopic structures of the fungus growing on MEA were examined under a Zeiss Axiophot compound light microscope. Color of cultures was described using Munsell soil color charts (Munsell 2000). Voucher specimens were deposited in the Herbarium of the Korean Forest Research Institute, Seoul, South Korea (HKFRI), and cultures were conserved in Jengeup-si, Jeollabuk-do, South Korea (KCTC).

For DNA extraction, PCR, and DNA sequencing, fungal cultures grown on MYEA (2% malt extract, 0.2% yeast extract, and 1.5% agar) at room temperature for 14 days were used. Methods for DNA extraction, PCR (including primers), and sequencing of ITS rDNA followed Yun & Rossman (2011). ITS sequences were deposited in GenBank. The ITS sequences for our isolates along with other known *Tubakia* species were preliminarily aligned using Clustal X version 2.1 under default settings (Thompson & al. 1997). The alignment was edited using Mesquite 3.03 beta 2 (Maddison & Maddison 2015*). Greeneria uvicola* was used as outgroup for *Tubakia* since this species was among the closest to genus *Tubakia* based on BLAST search at the National Center for Biotechnology information (NCBI; https://www.ncbi.nlm.nih.gov). Maximum parsimony analysis (MP) was conducted using PAUP version 4.0b10 (Swofford 2002), using a heuristic search with the starting tree obtained via stepwise addition with random addition of 1000 replicates, tree–bisection–reconnection (TBR) as the branch swapping algorithm, and MULTREES off. All characters were unordered, whereas equal weights and gaps were treated as a fifth character. Stability of the clades (bootstrap) was assessed with 1000 replicates using the same MP settings. Bootstrap values greater than or equal to 70% were considered significant (Hillis & Bull 1993). Pathogenicity tests of *Tubakia koreana* on *Quercus acutissima* were performed using inoculation protocols modified from those of Harrington & al. (2012).

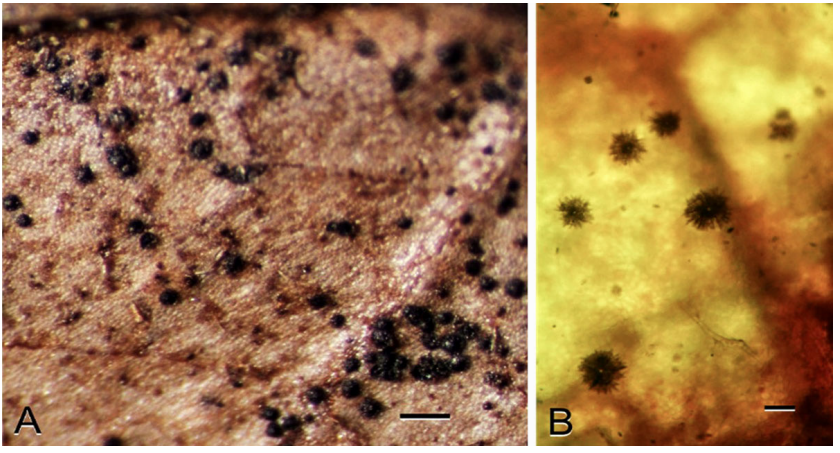

Fig. 1. *Tubakia koreana* (HKFRI4072) on *Quercus acutissima*:
Sign of the blight, black crustose conidiomata. Scale bars: A = 1 mm; B = 0.5 mm.

Taxonomy

Tubakia koreana H.Y. Yun, **sp. nov.** Plates 1. 2

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Differs from *Tubakia dryina* by its brown colony color and its bigger spore size.

Type: South Korea, Gyeonggi: Mt. Geom-dan, Hanam-si, Sinjang-dong, living tree of *Q. mongolica* Fisch. ex Ledeb., 18 July 2012, HY Yun, HY946 (**Holotype**, HKFRI 4073; ex-type culture, KCTC 46072; GenBank KP886837).

Etymology: *koreana* refers to the country where the species was collected.

The fungus was consistently isolated from leaves with blight symptoms produced in nature and by artificial inoculation. Small crustose conidiomata with radiate scutella formed on the necrotic tissues of mostly the upper side or occasionally on both sides of leaf surfaces. The fungal colonies fully grown on malt extract agar (MEA) at 25°C were felt-like and very pale brown to brown in color with concentric rings of dense aerial mycelium and a scalloped margin. Scutella that formed on leaf surfaces were 60–180 × 55–120 µm, membranous, composed of thick-walled, pale brown to brown, septate hyphae radiating, typically bifurcating up to three times from a central disc that consisted of a single hyaline cell towards the margin, acute and cornuted at the margin that was fringed and unattached to the substrate. Conidia were 8.5–14.5 × 6–10 µm, blastic, subglobose, broadly

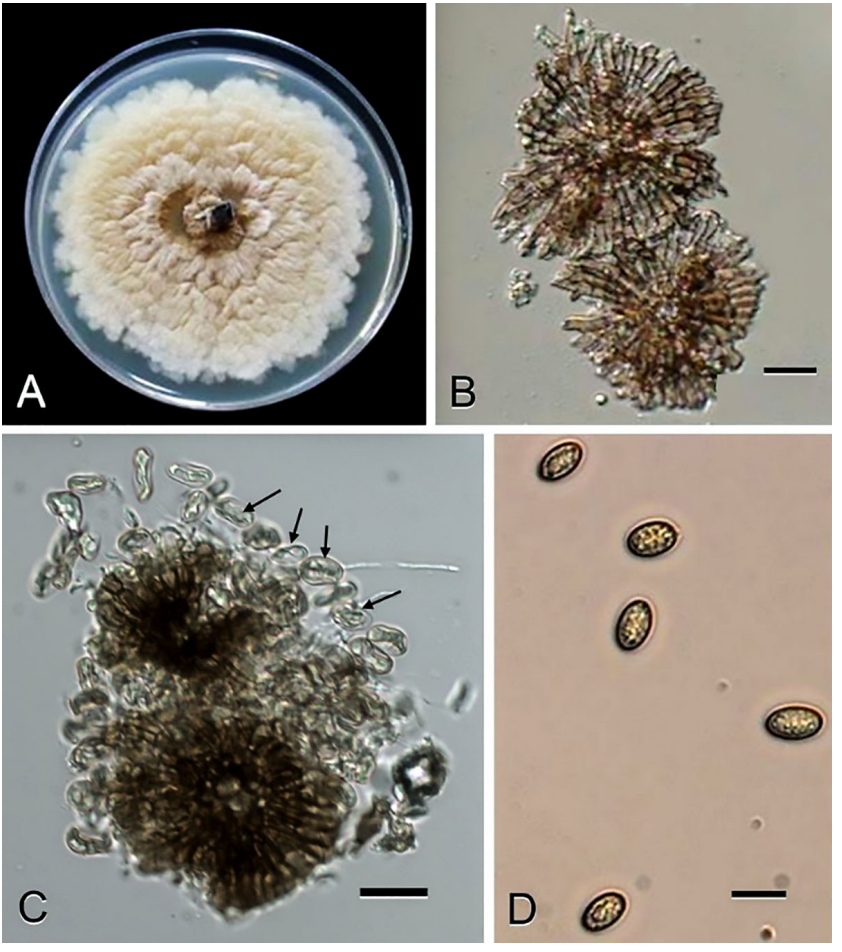

Fig. 2. *Tubakia koreana* (HKFRI4073). A. Colony grown on malt extract agar at 25°C for 14 days; B. Scutella; C. Scutella and conidia (arrows); D. Conidia. Scale bars: B = 50 μm; C = 20 μm; D = 10 μm.

ellipsoid to ellipsoid, and hyaline. They were turning pale yellowish brown, and the walls were smooth. Microconidium was not observed.

Additional specimens examined: **SOUTH KOREA**: **Chungbuk**, **Buk-myeon,** Inje-gun, living tree of *Quercus mongolica*, 5 July 2012, HY Yun, HY882 (HKFRI4083); **Jeungpyeong-gun,** Jeungpyeong-eup, Miam-ri, living tree of *Q. acutissima* Carruth., 18 July 2012, HY Yun, HY1095 (HKFRI4090, KCTC46103), HY1096 (HKFRI4091,

KCTC46104), HY1098 (HKFRI4081, KCTC46105); living tree of *Q. serrata* Murray, 18 July 2012, HY Yun, HY1097 (HKFRI4082). **Gangwon**, **Wonju-si,** Halla Univ., Heungeop-myeon, Heung-eop-ri, living tree of *Q. mongolica*, 6 October 2012, HY Yun, HY134 (HKFRI4086); **Sokcho-si,** Mt. Seorak, Seorak-dong, living tree of *Q.* × *alienoserratoides* T.B. Lee, 28 October 2011, HY Yun, HY719 (HKFRI4071, KCTC46044, GenBank KP886836); Seoraksan National Park, Seorak-dong, living tree of *Q. acutissima,* 31 Aug. 2009, HY Yun, US227 (HKFRI4085); **Gyeonggi**, **Namyangju-si,** Mt. Un-gil, Songchon-ri, Joan-myeon, on living tree of *Q. mongolica,* 27 July 2012, HY Yun, HY989V (HKFRI4089, KCTC46092); **Jeonbuk**, **Muju-gun,** Mt. Deog-yu, Samgong-ri, Seolcheon-myeon, living tree of *Q. mongolica*, 10 July 2012, HY Yun, HY190 (HKFRI4070; KCTC46031; GenBank KP886834); **Jeonnam, Gwangyang-si,** Ongnyong-myeon, living tree of *Q. acutissima*, 24 February 2012, HY Yun, HY107 (HKFRI4084); **Seoul**: **Seoul,** Mt. Kwan-ak, Gwanak-gu, Daehak-dong, living tree of *Quercus acutissima*, 23 June 2012, HY Yun, HY816 (HKFRI4072, KCTC46051, GenBank KP886835).

Discussion

Initial symptoms were small pale-brownish and dehydrated areas on leaves that spread through leaf veins and blades until the entire leaf or large areas were blighted with necrotic mid and lateral veins. Those symptoms sometimes preceded premature defoliation. The mycological characteristics of the present pathogen were distinct from those of other *Tubakia* species; i.e., colony color (very pale brown to brown) differing from *T. dryina* (Sacc.), B. Sutton (light gray), *T. iowensis* (light and dark gray), and *T. seoraksanensis* (whitish to pale yellow); conidial size smaller than *T. seoraksanensis*; and an absence of microconidia unlike their occasional presence in *T. iowensis* (Harrington & al. 2012, Yun & Rossman 2011). Also, ITS rDNA sequence analysis of the four isolates from diseased oak trees showed they had identical sequences. In addition, phylogenetic analysis placed this fungus in a monophyletic lineage independent of other *Tubakia* species (Plate 3).

Maximum likelihood tree comparison revealed it is a unique monophyletic clade, distinct from other *Tubakia* species. Also, the observed symptom of severe necrosis is different than the symptom of leaf spots observed in *T. japonica*. The Korean *Tubakia* species has much smaller conidia than *T. japonica*. These observations all suggest that the causal agent of oak leaf blight in the present studies is a new *Tubakia* species with different mycological and genetic characteristics from other *Tubakia* species distributed worldwide. The species epithet is named *koreana* to indicate a pathogen that is prominent throughout the mountains of South Korea. The disease caused by this fungus should be named oak leaf blight,

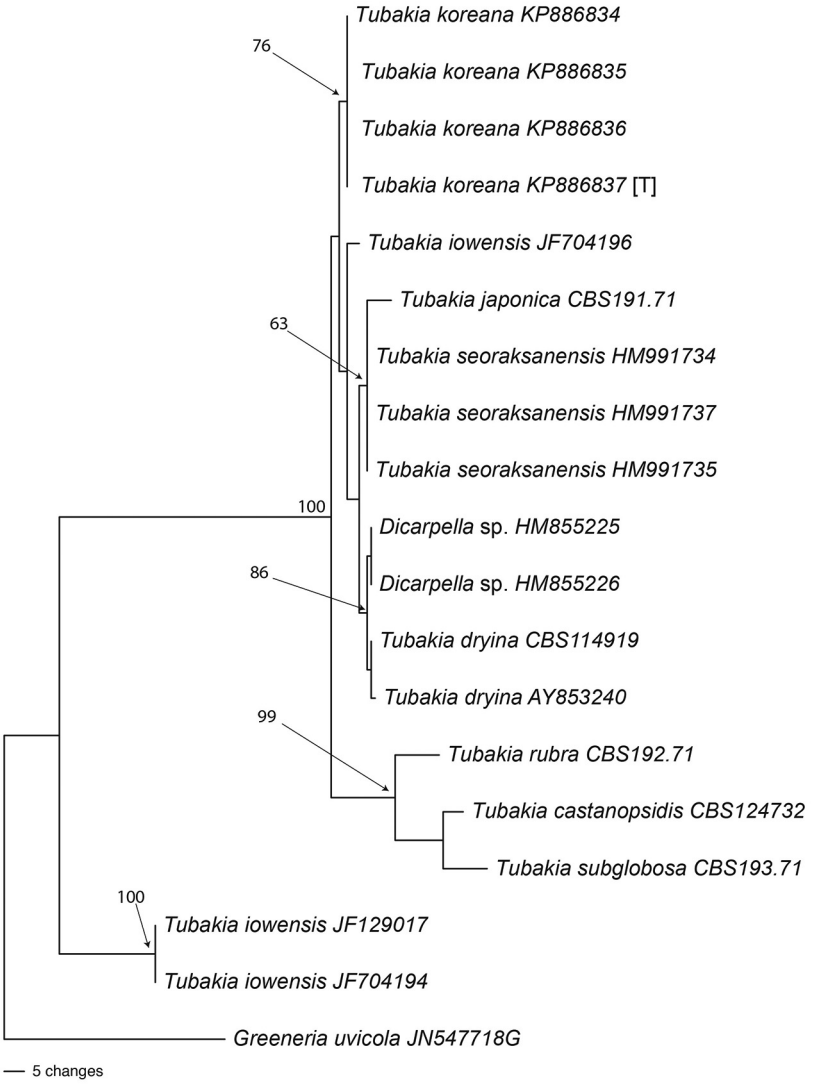

Fig. 3. Maximum likelihood tree produced from selected ITS rDNA sequences, showing the relations of *Tubakia koreana* isolates with other *Tubakia* species, [T]: holotype. The tree was obtained using PhyML 3.0. Numbers above the branch points are bootstrap values obtained from 1000 replicates.

rather than oak blight, since twig and branch dieback symptoms were rarely found, as in the bur oak blight reported in the U.S.A. (Harrington & al. 2012). In pathogenicity tests, leaf blight symptoms similar to naturally occurring symptoms developed on all oak trees wound-inoculated (mid vein) with conidial suspensions of *T. koreana* 15 days after inoculation; the most severe symptoms were observed on *Q. acutissima*. Oak trees are common tree species in Korea. Therefore, this disease could be a potential threat to the stable ecological succession of Korean forest vegetation.

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